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**No major role for glutathione S-transferase gene polymorphisms in sensitization to para-phenylenediamine and other xenobiotics; an association study and meta-analysis**

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It is our personal practice to utilize the antibiotic-containing local anaesthetic in all our surgical procedures given that we routinely perform large numbers of complex reconstructions at our institution. Individual surgeons may, however, prefer to reserve the use of intra-incisional antibiotic for higher-risk groups such as diabetic patients or the elderly or in prolonged surgical reconstructions. Given the small quantities of antibiotic used, coupled with the method of delivery, in our experience antibiotic resistance has not been a problem.

We have found the use of a buffered lidocaine solution (with the addition of intra-incisional antibiotic if so desired) to be safe, convenient and effective during dermatological surgery. We thus describe what we believe to be an efficient, labour-saving method of preparation. Given the ongoing financial constraints globally and within the National Health Service in the U.K., any method of maximizing efficiency and, as a consequence, productivity (without compromising the standard of care received by our patients) should be given careful consideration.

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## No major role for glutathione S-transferase gene polymorphisms in sensitization to para-phenylenediamine and other xenobiotics: a study of association and a meta-analysis

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MADAM, para-Phenylenediamine (PPD), an important contact allergen, may cause severe allergic contact dermatitis (ACD).

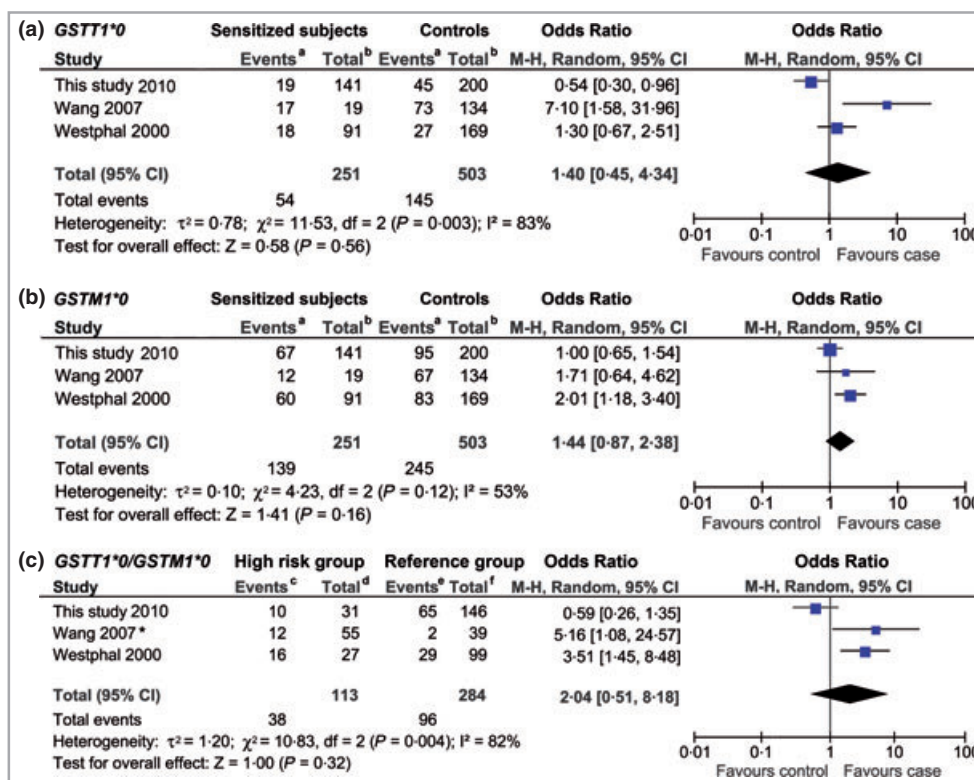
PPD is susceptible to auto-oxidation, resulting in reactive oxygen species (ROS) formation.<sup>1</sup> It has been found that oxidative stress from ROS may play an important role in the sensitization phase of ACD to PPD.<sup>1</sup> Glutathione S-transferases (GSTs) are known for their detoxifying role through scavenging ROS.<sup>2</sup> However, human GST genes display polymorphisms which are likely to contribute to interindividual differences in responses to xenobiotics.<sup>3</sup> By performing an association study as well as a meta-analysis, we examined the role of GST polymorphisms in sensitization to PPD and other xenobiotics. Entire gene deletion results in the null-alleles GST ( $\theta$ ) T1\*0 and GST ( $\mu$ ) M1\*0, subsequent absence of enzymatic activity and therefore, 'high risk'. For the GST pi-1 gene (GSTP1), a single nucleotide A to G substitution at position 313 is known.<sup>4</sup> The most common GSTP1\*313AA was taken as the reference allele. To determine a possible synergistic effect, the combined GSTT1/GSTM1 genotype was considered. Genotyping was performed by a real-time polymerase chain reaction (PCR) assay. The association analysis used a German case-control set, containing 150 cases and 202 controls. After approval by the ethics committee, participants gave written informed consent. For meta-analysis we identified two papers. Wang et al. (2007) studied sensitization to chromate, whereas Westphal et al. (2000) examined sensitivity to thimerosal.<sup>5,6</sup> Although these studies concern different xenobiotics, we assumed that they follow the same detoxification pathway.<sup>5,6</sup> Including those in our study we analyzed 251 patients with sensitivity to a xenobiotic (PPD, chromate or thimerosal) and 503 control subjects altogether. GSTT1\*0 was significantly more frequent in controls (22.5%) compared with sensitized subjects (13.5%), yielding an odds ratio (OR) of 0.54 [95% confidence interval (CI) 0.30–0.96,  $P = 0.04$ ] (Table 1). Neither GSTM1\*0, nor GSTT1\*0/GSTM1\*0 was significantly associated with sensitization to PPD (Table 1). GSTP\*313 genotypes were not significantly different in sensitized subjects compared with controls (Table 1). In the meta-analysis no significant relationship was found either between GSTT1\*0 or GSTM1\*0, or between GSTT1\*0/GSTM1\*0 and sensitized subjects (Fig. 1).

Contrary to our a priori hypothesis we observed a protective effect for GSTT1\*0. This finding is in contrast to that reported by Westphal et al. (2000) and Wang et al. (2007) who observed GSTT1\*0 more frequently in sensitized subjects.<sup>5,6</sup> To accommodate this variation across different studies, we performed a meta-analysis, which found no association of GSTT1\*0 and sensitization overall (Fig. 1). For GSTM1\*0, both we and Wang et al. (2007)<sup>5</sup> found no association with sensitization (Table 1). In contrast, Westphal et al. (2000)<sup>6</sup> found GSTM1\*0 significantly more frequent among sensitized subjects. Meta-analysis of all three studies found no difference in the frequency of GSTM1\*0 between sensitized subjects and controls (Fig. 1). Our association study on the GSTP1 polymorphism and sensitization to PPD yielded no statistical relationship (Table 1); possibly due to the controls deviating slightly from Hardy–Weinberg equilibrium (HWE) ( $P = 0.02$ ). For 'high-risk' profiled patients, i.e. GSTT1\*0/GSTM1\*0

**Table 1** Association between the glutathione S-transferase theta-1 (GSTT1), mu-1 (GSTM1), combined GSTT1/GSTM1 and GST pi-1 (GSTP1) polymorphisms and para-phenylenediamine (PPD)-sensitized subjects and controls

Genotype		Sensitized subjects <sup>a</sup> n = 141 <sup>b</sup> (%)	Controls n = 200 <sup>b</sup> (%)	OR (95% CI)
GSTT1 <sup>c</sup>	Present	122 (86.5)	155 (77.5)	1 (referent)
	Absent	19 (13.5)	45 (22.5)	0.54 (0.30–0.96) <sup>e</sup>
GSTM1 <sup>c</sup>	Present	74 (52.2)	105 (52.2)	1 (referent)
	Absent	67 (47.5)	95 (47.5)	1.00 (0.65–1.54)
GSTT1/GSTM1 <sup>c</sup>	Present/present	65 (46.1)	81 (40.5)	1 (referent)
	Absent/present	9 (6.4)	24 (12.0)	0.46 (0.20–1.08)
	Present/absent	57 (40.4)	74 (37.1)	0.95 (0.60–1.54)
	Absent/absent	10 (7.1)	21 (10.5)	0.59 (0.26–1.35)
GSTP1 <sup>d</sup>	Ile-Ile	65 (46.4)	87 (47.0)	1 (referent)
	Ile-Val	57 (40.7)	69 (37.3)	1.11 (0.69–1.78)
	Val-Val	18 (12.9)	29 (15.7)	0.83 (0.43–1.62)

CI, confidence interval; OR, odds ratio. <sup>a</sup>Sensitization was determined by patch testing; reading was performed according to the International Contact Dermatitis Research Group (ICDRG) guidelines. <sup>b</sup>The GSTP1 genotype has been genotyped successfully in 140 sensitized subjects and 185 controls. <sup>c</sup>'Present' represents the homozygous reference genotype (i.e. carriers), 'absent' represents the homozygous deleted genotype (i.e. noncarriers). <sup>d</sup>'Ile-Ile' represents the GSTP1\*313AA genotype, 'Ile-Val' represents the GSTP1\*313AG genotype, 'Val-Val' represents the GSTP1\*313GG genotype. <sup>e</sup>P-value = 0.036.



**Fig 1.** Meta-analysis: effect of glutathione S-transferase theta-1-null (GSTT1\*0), mu-1-null (GSTM1\*0) and combined GSTT1\*0/GSTM1\*0 genotypes on sensitization to xenobiotics. Single odds ratios (ORs) of the three studies involved as well as the total (i.e. pooled) ORs and the overall effect (Z-test) are shown. The 95% confidence interval (CI) of the pooled ORs was estimated using a Mantel-Haenszel (M-H) random effects model; heterogeneity among studies was calculated using  $\tau^2$ ,  $\chi^2$  and  $I^2$  tests; all implemented in Review Manager, version 5.0 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). A P-value < 0.05 was considered statistically significant. (a) GSTT1\*0 was considered the risk allele. (b) GSTM1\*0 was considered the risk allele. (c) The GSTT1+/GSTM1+ (i.e. combined presence) genotype was used as a reference. The GSTT1\*0/GSTM1\*0 (i.e. combined deletion) was considered the highest risk group. \*Wang et al. (2007) did not report the combined genotype in their paper, but provided their raw data upon request. <sup>a</sup>Number of carriers of the high-risk allele. <sup>b</sup>Total number of sensitized subjects or controls. <sup>c</sup>Number of sensitized subjects within the high-risk group. <sup>d</sup>Total number of subjects in the high-risk group. <sup>e</sup>Number of sensitized subjects within the reference group. <sup>f</sup>Total number of subjects in the reference group.

carriers, we found no association with sensitization to PPD (Table 1). Contrarily, analysis of the data of Wang et al. (2007) did show a significant effect of the combined deletion, as did that of Westphal et al. (2000). In conclusion, the current data have not shown a synergistic effect of GSTT1\*0/GSTM1\*0 in predisposition to sensitization. The discrepancy found in our association analysis cannot be explained thus far. However, especially given the borderline significant association for GSTT1\*0, additional studies with a larger sample size might help. The very different findings between our study and the other two studies can possibly be explained by the fact that detoxification of the different xenobiotics might be dependent on additional factors and hence cannot solely be attributed to the examined GST genes. In fact, this is true for PPD which is known also to be acetylated by N-acetyltransferases.<sup>7</sup> Moreover, major ethnic differences in frequency distributions of the GST deletions exist, which may at least account for the considerable difference found between our study and Wang's, as GSTT1\*0 is found most frequently in East Asia.<sup>8</sup> One limitation of our study is a possible misclassification due to the genotyping assay applied which only discriminated between carriers and noncarriers, while a relevant gene-dosage effect has been described.<sup>8</sup> Also, the previously described linkage disequilibrium between GSTT1 and GSTT2B polymorphisms in Caucasians, may have had a confounding effect.<sup>9</sup> Therefore, it may be necessary to evaluate the GSTT2B polymorphism in order to assess accurately the associations between GSTT1\*0 and sensitization. Like our results, meta-analysis of relationships between GST polymorphisms and cancers often yielded inconsistent and conflicting results.<sup>10</sup> Furthermore, GST genes have been suggested to have disease-modifying rather than disease-causing effects with a weak biological impact.<sup>4</sup> This might explain the lack of association found in sensitization studies. In conclusion, our data as well as others suggest that the common genetic polymorphisms in GSTs may not play a major role in predisposition to sensitization.

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## Treatment of cutaneous sarcoid with topical gel psoralen and ultraviolet A

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MADAM, Cutaneous manifestations of sarcoidosis occur in 25–30% of patients and are typically difficult to manage. Sarcoid commonly presents as reddish-brown papules, nodules or plaques. The pathognomonic lesion is lupus pernio, a variant that is more prevalent in Afro-Caribbean subjects, tends to persist and results in significant facial disfigurement. Less common presentations include hypopigmentation, subcutaneous nodules, alopecia and ulceration.

We report a series of six patients with cutaneous sarcoid successfully treated with topical gel psoralen and ultraviolet A